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THE ROLE OF MYOSIN TAIL IN PRESSURE-INDUCED GELATION OF MYOSIN

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Introduction:

Myosin is a major protein in myofibril and it plays important roles in binding or water holding in meat products. Myosin molecule consists of two heads and a tail. The heads contain actin-binding sites and ATPase sites, and the tails are responsible for filament formation. Myosin filaments dissociate into monomers by increasing salt concentration. Both of myosin filaments and monomers form gels by heating. When high hydrostatic pressure is applied to myosin filaments, they also form a gel [1]. On the other hand, monomeric myosins at high salt concentration form aggregates, which are formed through head-to-head interaction, and never form a gel at least up to 500 MPa [2, 3]. Recently, we found that pressurized monomeric myosins form a gel by gradual lowering of salt concentration by dialysis. We temporarily call this type of gel as a dialysis-induced gel in this study. The present study was performed to clarify the roles of tail portion of myosin molecule in pressure-induced myosin filament gel and dialysis-induced gel as well.

Materials and Methods:

Myosin was extracted from rabbit skeletal muscle and purified by DEAE-Sephadex column chromatography. Myosin filaments in 0.1 M NaCl and monomeric myosin in 0.5 M NaCl were pressurized up to 500 MPa for 15 min. The pressurized monomeric myosin was then dialyzed against 0.1 M NaCl and 20 mM K phosphate (pH 6.0) to achieve gradual decrease of NaCl concentration. Pressure treated myosins, which are gel or suspension, were put into a dialysis tubing and dialyzed against 0.3–0.5 M NaCl and 20 mM K phosphate (pH 6.0) to examine solubility. Turbidity of solubilized fraction was measured as an absorbance at 350 nm. Microstructure of the gel was observed by a scanning electron microscope. Solubilized fraction by dialysis against high salt buffer was rotary shadowed and observed by a transmission electron microscope.

Results and Discussion:

Myosin filaments in 0.1 M NaCl at pH 6.0 formed a gel by pressurization above 200 MPa. While monomeric myosins in 0.5 M NaCl did not form a gel, they gelled by gradual lowering of NaCl concentration to 0.1 M by means of dialysis. We examined morphological and biochemical properties of these two types of pressure-assisted gels.

Microstructure of gel

Pressure-induced gel of myosin filaments in 0.1 M NaCl showed a fine and continuous network structure. In contrast, the network of dialysis induced gel was coarse and porous, and the connection of strands was discontinuous. Sensory test indicated that the dialysis-induced gel was apparently soft and fragile than the pressure induced myosin filament gel in 0.1 M KCl.

Turbidity of solubilized fraction of gel

Pressure treated myosin filaments in 0.1 M NaCl, and pressurized and then dialyzed monomeric myosin were dialyzed against 0.3–0.5 M NaCl. Turbidity of solubilized fraction after dialysis is shown in Fig. 1. In case of pressure treated myosin filaments, gelation did not occur at 100 MPa, while gel was formed above 200 MPa. When pressure treated myosin filaments were dialyzed against 0.3 M NaCl, slight decrease in turbidity was observed in the 100 MPa treated sample, and the gels formed at 200 and 250 MPa were partially solubilized by dialysis and those turbidities increased with applied pressure. The gels formed at 300–500 MPa were hardly solubilized by dialysis against 0.3 M NaCl. When pressurized myosin filaments were dialyzed against 0.4–0.5 M NaCl, all the gels were solubilized, and the turbidities of myosin filaments pressurized below 200 MPa decreased to almost the same level of monomeric myosin. This indicates dissociation of filaments with increasing salt concentration through dialysis.

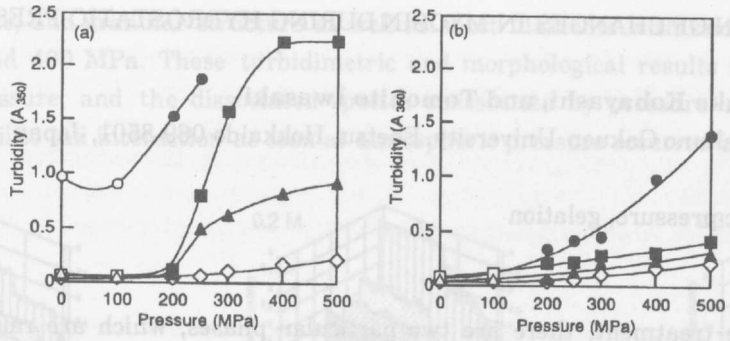

Fig. 1. Changes in turbidity (A_{350}) of solubilized gel by dialysis against 0.3 – 0.5 M NaCl.
(a), pressure-induced gel: (b), dialysis-induced gel. ◇, pressurized myosin monomer in 0.5 M NaCl. ○, □, and △ are 0.3, 0.4, and 0.5 M NaCl, respectively. Applied pressure was 100–500 MPa for 15 min. Open symbols indicate the myosin which were not gelled and closed symbols are gelled myosin.

Morphology of solubilized fraction of dialysis-induced gel

Morphology of solubilized fraction of dialysis-induced gel of pressurized monomeric myosin was observed with a transmission electron microscope (Fig. 2). Unpressurized myosin molecule consists of two heads and a tail (a). Pressurized myosins in 0.5 M NaCl at 500 MPa for 15 min showed daisy-wheel-like aggregates in which myosin molecules were associated through head to head interaction (b). Although such aggregates were also observed in the solubilized fraction of dialysis-induced gel, the connected aggregates were distinguished (c, arrowhead). These morphological observation suggests that dialysis-induced gel is formed through tail to tail interaction among the aggregates, and the tails which are expanded from the aggregated head core retains their self-association capability even after pressure treatment at least up to 500 MPa.

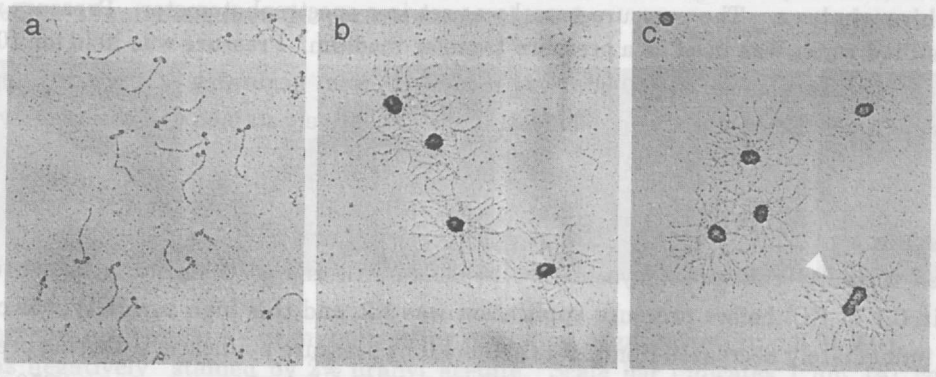

Fig. 2. Transmission electron micrographs of solubilized fraction of dialysis-induced gel from pressurized monomeric myosin (A); unpressurized myosin, (B); pressurized myosin in 0.5 M NaCl at 500 MPa for 15 min, (C); solubilized fraction of dialysis-induced gel from pressurized monomeric myosin.

Conclusion:

Pressurized monomeric myosin in 0.5 M NaCl formed a gel by gradual lowering of salt concentration through dialysis against 0.1 M NaCl. This type of gel is formed by connecting pressure-induced daisy-wheel-like aggregates through tail to tail interaction. Pressure induced myosin filament gel in 0.1 M NaCl is formed through head to head interaction among the filaments. The tail of myosin molecule retains the ability of self-association even after pressure treatment up to 500 MPa.

References:

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